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cDNA Cloning and Chromosome Mapping of Human Dihydropyrimidine Dehydrogenase, an Enzyme Associated with 5-Fluorouracil Toxicity and Congenital Thymine Uraciluria*

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Hiroshi Yokota‡, Pedro Fernandez-Salguero, Hirokazu Furuya§, Keming Lin¶, O. Wesley McBride¶, Beate Podschun‖, Klaus D. Schnackerz‖, and Frank J. Gonzalez**

From the Laboratory of Molecular Carcinogenesis and ¶Laboratory of Biochemistry, National Cancer Institute, Bethesda, Maryland 20892 and ‖Theodor-Boveri-Institut fuer Biowissenschaften, Physiologische Chemie I, Am Hubland, D-97074 Wuerzburg, Germany

The pig and human dihydropyrimidine dehydrogenase (DPD) cDNAs were cloned and sequenced. The pig enzyme, expressed in *Escherichia coli*, catalyzed the reduction of uracil, thymine, and 5-fluorouracil with kinetics approximating those published for the enzyme purified from mammalian liver. DPD could be expressed in significant quantities only when uracil was added to the bacterial growth medium. The pig and human enzymes contained 1025 amino acids and calculated M_r = 111,416 and 111,398, respectively. Conserved domains corresponding to a possible NADPH binding site and FAD binding site were found in the NH_2-terminal half of the proteins and two motifs of putative [4Fe–4S] binding sites were found near to the carboxyl terminus of the enzyme. The latter corresponds to the labile COOH-terminal fragment previously shown to contain the iron sulfur centers. A sequence encompassing a peptide corresponding to the uracil binding site was found between the NADPH/FAD-containing NH_2-terminal portion of the protein and the iron-sulfur binding sites near to the COOH terminus. Thus, the DPD appears to be derived from at least three distinct domains. The *DPYD* gene was localized to the centromeric region of human chromosome 1 between 1p22 and q21.

Dihydropyrimidine dehydrogenase (DPD, EC 1.3.1.2)[1] is the initial and rate-limiting enzyme in the three-step pathway of uracil and thymine catabolism and in the pathway leading to the formation of β-alanine (Wasternack, 1980). DPD is also the principal enzyme involved in degradation of the chemotherapeutic drug 5-fluorouracil, which acts by inhibiting thymidylate synthase (Heggie *et al.*, 1987; Chabner and Myers, 1989; Diasio *et al.*, 1988; Diasio and Harris, 1989; Grem, 1990). The activity of DPD is highly correlated with 5-fluorouracil pharmacokinetics (Goldberg *et al.*, 1988; Harris *et al.*, 1990; Fleming *et al.*, 1992). The efficacy of this agent might be related to plasma levels of the drug which are inversely related to the level of DPD activity (Iigo *et al.*, 1988).

Patients exhibiting severe toxicity when administered 5-fluorouracil were shown to have low DPD activity (Tuchman *et al.*, 1985; Diasio *et al.*, 1988; Harris *et al.*, 1991; Fleming *et al.*, 1993; Houyau *et al.*, 1993; Lyss *et al.*, 1993). Studies in families suggest that this deficiency follows an autosomal recessive pattern of inheritance (Diasio *et al.*, 1988). DPD is associated with inherited disorders of pyrimidine metabolism, clinically termed thymine-uraciluria (Bakkeren *et al.*, 1984). The clinical symptoms have been described as nonspecific cerebral disfunction and the defect has been associated with psychomotor retardation, convulsions, and epileptic conditions (Berger *et al.*, 1984; Wadman *et al.*, 1984; Wilcken *et al.*, 1985; van Gennip *et al.*, 1989; Brockstedt *et al.*, 1990; Duran *et al.*, 1985). Eleven patients have been found in the Netherlands alone, thus suggesting that total or partial deficiency in DPD may not be as rare a disorder as previously assumed (van Gennip *et al.*, 1993). Genotyping assays to determine subjects that are lacking sufficient DPD activity would be of use in prenatal and neonatal diagnosis and for prescreening cancer patients prior to 5-fluorouracil therapy.

DPD has been purified from liver tissue of rats (Shiotani and Weber, 1981; Fujimoto *et al.*, 1991), pig (Podschun *et al.*, 1989), cattle (Porter *et al.*, 1991), and human (Lu *et al.*, 1992). The pig enzyme contains flavins and iron-sulfur prosthetic groups and exist as a homodimer with a subunit M_r of about 107,000 (Podschun *et al.*, 1989). Since the enzyme exhibits a nonclassical two-site ping-pong mechanism, it appears to have distinct binding sites for NADPH/NADP and uracil/5,6-dihydrouracil (Podschun *et al.*, 1990). A model for DPD activity incorporating an acid base catalytic mechanism has been proposed (Podschun *et al.*, 1993).

In an effort to determine the mechanism of the DPD deficiency in humans and to analyze the molecular details of enzyme, the pig and human cDNAs were cloned and sequenced. The human *DPYD* gene was mapped and the pig enzyme was expressed and kinetically characterized using *Escherichia coli*.

MATERIALS AND METHODS

Cloning of the Pig and Human DPD cDNAs—Total RNA was isolated from frozen pig liver by use of the method of Chirgwin *et al.* (1979) except that CsCl was replaced with Cs-trifluoroacetic acid (Pharmacia Biotech Inc.). The RNA was twice extracted with a phenol-chloroform emulsion and ethanol precipitated prior to use. Poly(A)-containing RNA was isolated by oligo(dT)-cellulose chromatography (Aviv and Leder, 1977) and used as a template to prime synthesis of cDNA. The cDNA

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) U09179 and U09178, for pig and human cDNAs, respectively.

‡ Present addresses: School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069, Japan.

§ Present address: Dept. of Neurology, Neurological Institute, Kyushu University 60, Fukuoka 812, Japan.

** To whom correspondence should be addressed: National Institutes of Health, Bldg. 37, Rm. 3E-24, Bethesda MD 20892. Tel.: 301-496-9067; Fax: 301-496-8419.

[1] The abbreviations used are: DPD, dihydropyrimidine dehydrogenase; DPYD, dihydropyrimidine dehydrogenase gene; NRAS, neuroblastoma RAS viral (v-ras) oncogene homolog; PGM1, phosphoglucomutase 1 gene; PBS, phosphate-buffered saline.

was made double-stranded and cloned into λgt22A using a kit supplied by Life Technologies, Inc. The DNA was packaged using the λ packaging system of the same vendor and the phage particles were plated on *E. coli* Y1090 r⁻. The library was screened using a polyclonal antibody against pig DPD (Podschun *et al.*, 1989). The partial cDNA obtained was used to rescreen the library on *E. coli* Y1088 by plaque hybridization to isolate a cDNA containing the complete protein reading frame. The cDNA was subcloned into the *Not*I-*Sal*I sites of the plasmid vector pSPORT (Life Technologies, Inc.). A region containing the coding sequence of the pig cDNA was used to screen previously amplified human liver cDNA libraries prepared in λgt11 (Yamano *et al.*, 1989). The human cDNA was isolated as three overlapping fragments and subcloned into the *Eco*RI site of pUC18. The three fragments were joined together using overlapping *Cla*I sites in pUC18 and the complete sequences of pig and human DPD cDNAs were determined using an Applied Biosystems 373A DNA sequencer using synthetic primers and fluorescent dye terminator chemistry. The oligonucleotides were made using an Applied Biosystems 380B DNA synthesizer and purified by filtration through a Centricon 10 filter (Millipore Corp.). Each base was determined at least once on both strands. DNA and deduced protein was analyzed using MacVector sequence analysis software (Intl. Biotechnology Labs, Inc., New Haven, CT).

Chromosome Localization of the DPYD Gene—The *DPYD* gene was localized to a specific human chromosome using the somatic cell hybrid strategy. Human-mouse and human-hamster cell lines were generated and characterized as described by McBride *et al.* (1982a, 1982b, 1982c, 1982d). The human chromosome content of each cell line was determined by standard isoenzyme analyses as well as by Southern analysis with probes from previously localized genes and, frequently, by cytogenetic analysis. Southern blots of hybrid cell DNA restriction digests on positively charged nylon membranes were prepared after (0.7%) agarose gel electrophoresis and hybridized at high stringency with ^{32}P-labeled probes under conditions allowing no more than 10% divergence of hybridizing sequences.

Preparation of Probes—Inserts representing the 3′ and 5′ coding regions of the human DPD cDNA were each labeled to high specific activity (>10^9 cpm/μg of DNA) by random hexamer primed DNA synthesis (Feinberg and Vogelstein, 1983).

Construction of the Expression Plasmid—The expression plasmid has been constructed in the vector pSE420 (Invitrogen Corp., San Diego, CA) using the pig DPD cDNA previously subcloned into the vector pSPORT. The cDNA contains an *Nco*I site coincident with the start codon (CCATGG) which was joined to the *Nco*I site in the vector that is in frame with the bacterial initiator Met. The pig DPD cDNA was inserted into pSE420 as an *NcoI*/*Afl*III fragment from pSPORT.

DPD Expression in E. coli—For each expression experiment, a single colony from a freshly made transformation of DH-5α cells with the expression vector was inoculated in LB broth and grown to stationary phase. A 1/100 aliquot from this culture was used to inoculate 250 ml of terrific broth containing 100 μg/ml ampicillin and supplemented with 100 μM of each FAD and FMN, 100 μM uracil, and 10 μM each of $Fe(NH_4)_2(SO_4)$ and Na_2S. Following a 90-min incubation at 29 °C, the *trp-lac* promoter in the expression vector was induced by the addition of 1 mM isopropyl-β-D-thiogalactopyranoside and the culture was incubated for an additional 48 h.

The cells were then sedimented, washed twice with 250 ml of phosphate-buffered saline (PBS) and resuspended in 45 ml of 35 mM potassium phosphate buffer (pH 7.3) containing 20% glycerol, 10 mM EDTA, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, and 2 μM leupeptin. The cell suspension was lysed at 4 °C with four 30-s bursts of a Heat Systems sonicator (model W 225-R at 25% of full power). The resultant lysate was centrifuged at 100,000 × *g* for 60 min at 4 °C. Solid $(NH_4)_2SO_4$ was then slowly added to the supernatant at 4 °C with gentle stirring to give a final concentration of 30% saturation. The precipitate was sedimented and the pellet containing expressed DPD was resuspended in 5 ml of 35 mM potassium phosphate buffer (pH = 7.3) containing 1 mM EDTA, 1 mM dithiothreitol, 2.5 mM magnesium chloride, and 0.1 mM phenylmethylsulfonyl fluoride. The protein solution was dialyzed at 4 °C for 36 h against 3 × 4 liters of buffer and stored at −70 °C until further use.

Catalytic Assay—DPD activity was determined at 37 °C by measuring the decrease in absorbance at 340 nm associated with the oxidation of NADPH to NADP⁺. The reaction mixture contained 28 mM potassium phosphate buffer (pH 7.3), 2 mM $MgCl_2$, 1 mM dithiothreitol, 60 μM NADPH, and the expressed DPD in a final volume of 1 ml. The measurements were carried out using an Aminco DW-2000 double beam spectrophotometer using a blank which contained the complete reaction mixture except substrate. The reactions were initiated by addition of substrate (uracil, 5-fluorouracil, or thymine). The catalytic activity was calculated as micromoles of NADPH oxidized/min and per mg of expressed DPD. Protein quantitations were determined using the bicinchronic (BCA) procedure from Pierce following the manufacturer's directions.

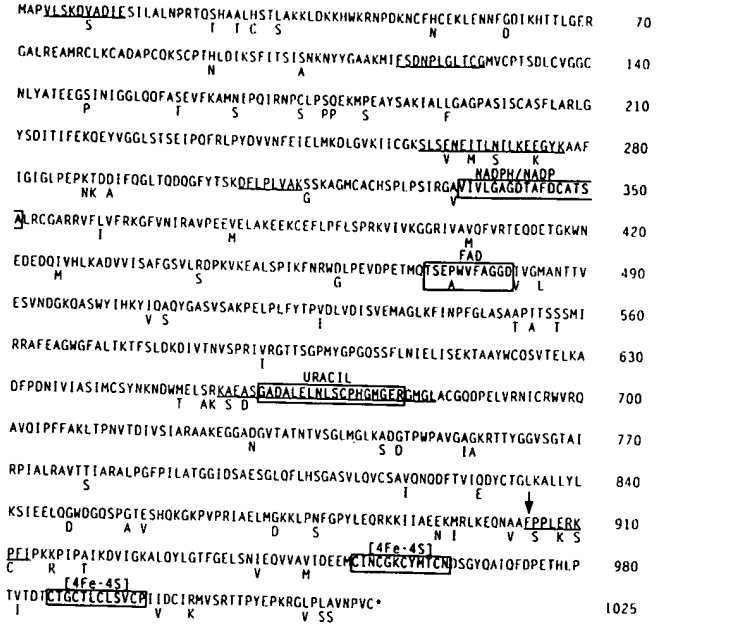

FIG. 1. **Comparison of the human and pig DPD cDNA-deduced amino acid sequences.** Only those amino acid residues of human DPD that differ from the pig sequences are shown *below* the pig DPD. The different motifs relevant for catalytic activity are enclosed in *boxes* and labeled as discussed in the text. Peptides sequenced in this study are *underlined*, and the site of cleavage to generate the 12-kDa [4Fe–4S] fragment is shown by a *vertical arrow*.

Analysis of cDNA-expressed DPD Protein

SDS-polyacrylamide gel electrophoresis was carried out following the method of Laemmli (1970) using 8% acrylamide slab gels. The SDS-polyacrylamide gels were transferred to a nitrocellulose membrane by electroblotting for 90 min at 1.5 mA/cm² (Towbin *et al.*, 1979). The membranes were blocked at room temperature using PBS containing 0.5% Tween 20 and 3% skim milk. After blocking, the membranes were incubated for 4 h at room temperature with rabbit anti-pig DPD polyclonal antibody dilute 200-fold in PBS. The membranes were washed three times in PBS containing 0.5% Tween 20 and rinsed twice with PBS prior to addition of alkaline phosphatase-labeled goat anti-rabbit IgG. Incubation was continued for 90 min, and the membranes were developed using the reagent 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (Kirkegaard & Perry Labs, Gaithersburg, MD).

RESULTS AND DISCUSSION

Isolation and Sequencing of Pig and Human cDNAs—Two partial pig cDNAs were isolated by screening 1 × 10^6 plaques from an unamplified λgt22A library. After verification by sequencing, a partial cDNA was used to rescreen 500,000 plaques; four cDNAs were isolated containing about 4.5 kilobase pairs. One of these were completely sequenced and found to encompass the full coding region of the protein (Fig. 1). The amino-terminal region agreed with that determined from the pig enzyme (Podschun *et al.*, 1989). The published sequence was preceded by Met, Ala, and Pro which probably corresponds to the initiation Met. A number of segments of amino acids previously sequenced were found in the cDNA-deduced protein (Fig. 1, *underlined*). These were determined by cyanogen bromide cleavage (residues 117–127) and trypsin cleavage (residues 260–277; 308–315; 656–682; 904–913) followed by high performance liquid chromatography separation and sequencing (data not shown). The first residue of the amino-terminal portion of the 12,000-dalton cleavage fragment from the pig

DPD is shown by a *verticle arrow* at residue 904. These data establish the pig DPD open reading frame of 1025 amino acids. The human cDNA-deduced sequence is identical to that of pig except where indicated in Fig. 1. The calculated molecular masses are 111,416 and 111,398 daltons for pig and human DPD, respectively. The poly(A) addition sequence of AAATAAA is found 17 base pairs upstream of a putative poly(A) tract cloned in the cDNA. This 3'-untranslated region was not isolated in the human cDNA clones.

The cDNA-derived protein sequences revealed the presence of a number of putative binding sites for known DPD cofactors. Recent EPR measurements on DPD from *Alcaligenes eutrophus* confirmed the existence of FMN, iron, and acid-labile sulfide, the latter two of which are indicative of iron-sulfur clusters (Schmitt *et al.*, 1994). The COOH-terminal 12-kDa peptide fragment purified from the pig DPD shows absorbance in the 500–600-nm region and contains eight iron and eight acid-labile sulfides (Podschun *et al.*, 1989). The binding site of iron-sulfur clusters contain Cys residues, a large number of which are found in the NH_2-terminal half of the protein. However, these do not exhibit the typical motif pattern seen in other well characterized iron-sulfur-containing proteins. In the COOH-terminal region of pig and human DPD are typical motifs C*XX*-C*XX*C*XXX*C*X* and C*XX*C*XX*C*XXX*CP for [4Fe–4S] clusters (Dupuis *et al.*, 1991) between residues 953 and 964 and residues 986 and 997, respectively. These lie within the 12-kDa iron-sulfur cluster-containing peptide (Podschun *et al.*, 1989).[2] No other [4Fe–4S] clusters were detected; however, other types of iron-sulfur clusters such as [2Fe–2S] might be possible.

A typical NADPH binding motif V*X*V*X*G*X*G*XX*G*XXX*A*XX*A (Wierenga *et al.*, 1985) was found beginning with Val-335, except that the Gly at position 10 is an Ala in pig and human DPD. A motif for FAD binding as reported by Eggink *et al.* (1990), T*XXXX*VFA*X*GD, was found in the NH_2-terminal region starting with Thr-471 and ending with Asp-481.

The putative uracil binding site of DPD was elucidated by incubating 5-iodouracil, a suicide inactivator of the bovine enzyme, and sequencing of the modified chymotryptic peptide (Porter *et al.*, 1991). The corresponding sequence obtained is located between Gly-661 and Arg-678 in the primary protein sequence. Thus, the functional domains of DPD can be arranged from the NH_2 terminus in the order NADPH/NADP-FAD-uracil-[4Fe–4S].

Localization of the DPYD Gene—The *DPYD* gene was localized to human chromosome 1 by Southern analysis of a panel of human/rodent somatic cell hybrid DNAs digested with *Eco*RI using a 3' coding cDNA fragment as probe (Table I). The gene segregated discordantly (≥14%) with all other human chromosomes. The 3' probe identified a series of bands in human DNAs ranging in size from 0.8 to 15 kilobases. All hybridizing human bands appeared to cosegregate indicating that these bands were all present on the same chromosome. The gene was then sub-localized on chromosome 1 by analysis of hybrids containing spontaneous breaks and translocations involving this chromosome. One human/hamster hybrid with a break between NRAS (1p12) and PGM1 (1p22) retained the telomeric portion of the chromosome 1 short arm but the *DPYD* gene was absent from this hybrid. Another human/hamster hybrid and a human/mouse hybrid each retained all, or nearly all, of the short arm of chromosome 1 including NRAS and all other short arm markers but all long arm markers were absent including a cluster of genes at 1q21 (trichohyalin, loricrin, and filaggrin); the human *DPYD* gene was present in both of these hybrids. Finally, one additional human/hamster hybrid retained a centromeric fragment of chromosome 1 with the breakpoints on the long arm and short arm proximal to 1q21 and proximal to 1p31, respectively, and human *DPYD* was present in this hybrid. These results indicate that the *DPYD* gene can be sublocalized to the region 1p22-q21. These results were confirmed by Southern analysis of the same panel of hybrids with a DPD 5' cDNA probe which detected 1.5-, 5.0-, 8.7-, and 11.6-kilobase bands in human *Eco*RI digests. Both probes were used to examine DNAs from ten unrelated individuals separately digested with 12 different restriction enzymes for restriction fragment length polymorphisms. However, no polymorphisms were detected. A large number of hybridizing bands were detected with both DPD probes and these bands cosegregated indicating that they are all localized to the centromeric region of human chromosome 1 (*i.e.* 1p22–q21). A number of cross-hybridizing hamster and mouse bands were also identified with these probes. These results are consistent with the interpretation that there may be a single reasonably large gene (spanning at least 80 kilobases) in each of these species, and all hybridizing bands arise from a single gene. However, we currently cannot exclude the possibility that the many hybridizing bands arise from a cluster of tandemly linked genes.

More precise localization of the gene will require *in situ* hybridization and/or genetic linkage analysis. It will be necessary to identify polymorphism(s) within, or flanking, the gene to facilitate linkage analysis and allow examination of families with genetic diseases for possible involvement of this gene. No

TABLE I
Segregation of dihydropyrimidine dehydrogenase gene with human chromosome 1

The human dihydropyrimidine dehydrogenase gene was detected by Southern blotting of a panel of *Eco*RI digested human/rodent somatic cell hybrid DNAs with a 1.8-kilobase (kb) human DPD 3' cDNA fragment. Ten hybridizing bands (0.8, 1.5, 2.2, 3.2, 3.4, 5.5, 6.0, 10.0, 11, and 15 kb) were detected in human DNAs with this probe, and most of these bands were resolved from 1.4-, 1.9-, 4.2-, 5.5-, 10.5-, and 16-kb or 2.6-, 4.4-, 7.5-, 8.9-, 10-, and 20-kb cross-hybridizing bands in hamster and mouse DNA digests, respectively. Detection of the human *DPD* gene is correlated with the presence or absence of each human chromosome in the group of somatic cell hybrids. Discordancy indicates the presence of the gene in the absence of the chromosome (+/–) or absence of the gene despite the presence of the chromosome (–/+), and the sum of these numbers divided by total hybrids examined (×100) represents the percent discordancy. The human/hamster hybrids consisted of 29 primary hybrids and 13 subclones (14 positive of 42 total) and the human/mouse hybrids represented 20 primary hybrids and 31 subclones (16 positive of 51 total). The gene could be sublocalized to 1p22–q21 by examination of hybrids containing specific translocations (see text).

Human chromosome	Gene/chromosome				Percent discordancy
	+/+	+/–	–/+	–/–	
1	30	0	0	63	0
2	22	8	5	58	14
3	20	10	13	50	25
4	27	3	30	33	35
5	21	9	5	58	15
6	29	1	21	42	24
7	19	11	24	39	38
8	18	12	19	44	33
9	7	23	7	56	32
10	13	17	7	56	26
11	19	11	12	51	25
12	22	8	18	45	28
13	16	14	19	44	35
14	17	13	28	35	44
15	19	11	29	34	43
16	15	15	23	40	41
17	26	4	32	31	39
18	21	9	26	37	38
19	23	7	6	57	14
20	24	6	17	46	25
21	20	10	39	24	53
22	19	11	14	49	27
X	18	12	28	35	43

[2] B. Podschun and K. D. Schnackerz, unpublished results.

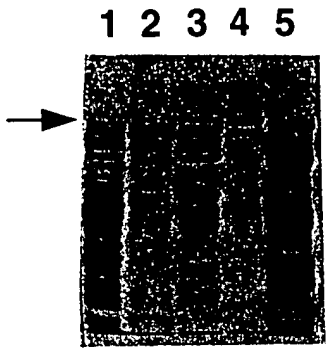

FIG. 2. **SDS-polyacrylamide gel analysis of cDNA-expressed pig DPD.** Total solubilized protein (10 μg) from cells containing the expression vector alone incubated with uracil (*lane 1*) and cells having the cDNA-containing vector incubated without (*lane 2*) and with uracil (*lane 3*) were subjected to electrophoresis, and the protein bands were stained with Coomassie Blue R-250. Purified pig liver DPD (5 μg) was co-electrophoresed (*lane 4*), and the position of migration of the intact enzyme is indicated by an *arrow*. *Lane 5* contains prestained molecular mass markers corresponding to phosphorylase *b* (105 kDa), bovine serum albumin (70 kDa), ovalbumin (43 kDa), and carbonic anhydrase (28 kDa).

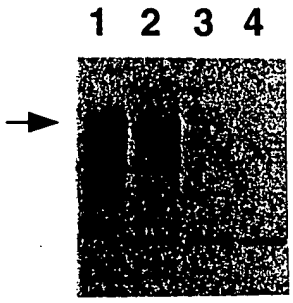

FIG. 3. **Western immunoblot analysis of cDNA-expressed pig DPD.** Ammonium sulfate-enriched fractions (10 μg) of lysates from cells containing the DPD cDNA incubated in the presence (*lane 2*) and absence (*lane 3*) of uracil and cells containing vector alone incubated in the presence of uracil (*lane 4*) were electrophoresed and transferred to nitrocellulose membrane, and the enzyme was stained using polyclonal antibody and alkaline phosphatase-conjugated goat anti-rabbit antibody. *Lane 1* contains 5 μg of purified DPD.

diseases have been localized to this chromosomal region which would likely involve this gene.

cDNA Expression of Pig DPD—The pig DPD was expressed in bacteria using the vector pSE420 which has a *trp-lac* promoter that is inducible by isopropyl-β-D-thiogalactopyranoside. Optimal expression was obtained when cells were grown between 26 and 30 °C. Growth at higher temperatures resulted in aggregation of the protein in inclusion bodies. A number of cofactors known to be associated with the enzyme were added to the medium; the most critical was uracil which resulted in over 5-fold higher levels of expression than in unsupplemented cells (Fig. 2). The expressed enzyme comigrated with the intact 107-kDa DPD purified from pig liver and reacted with rabbit polyclonal antibody directed against the pig enzyme (Fig. 3). DPD protein was undetectable in cells containing vector alone in the absence of cDNA (Figs. 2 and 3). The DPD purified from pig liver frequently has a second higher mobility band of about 12 kDa that results from a protease-labile site that liberates the iron sulfur-containing COOH-terminal fragment (Podschun *et al.*, 1989). The bacterial expressed enzyme is produced intact and could be significantly enriched from other *E. coli* proteins by a single ammonium sulfate fractionation. The other higher mobility proteins detected on the Western blots probably result from cross-reactivity of the antibody to bacterial antigens, since these proteins were also detected in cells containing vector alone. This finding is not suprising since the antibody was prepared using bacterial adjuvant. By use of the purified pig DPD as a standard, the level of expression in *E. coli* was estimated at 50 to 100 mg/liter of culture.

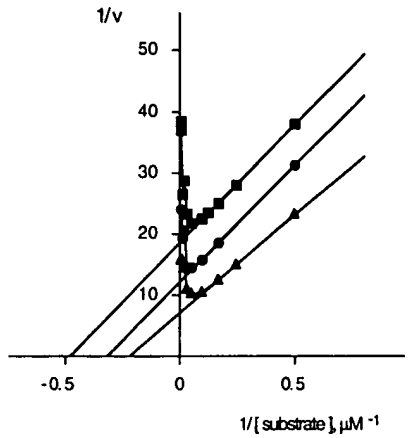

FIG. 4. **Kinetic analysis of expressed DPD.** Rates of metabolism of uracil (●), thymine (■), and 5-fluorouracil (▲) were determined by following NADPH reduction as a function of substrate concentration.

TABLE II
Comparison of the kinetic parameters of bacterial-expressed DPD with purified mammalian enzymes

Values are expressed as μM and μmol/min/mg of expressed DPD protein for K_m and V_{max}, respectively.

Substrate	Parameter	Expressed	Human[a]	Pig[a]	Rat[a]
Uracil	K_m	3.17	4.9	1.98	1.80
	V_{max}	0.084	0.6	0.33	0.69
5-Fluorouracil	K_m	4.57	3.3	5.50	NA[b]
	V_{max}	0.14	0.9	0.40	NA
Thymine	K_m	2.08	4.8	2.66	2.60
	V_{max}	0.053	0.7	0.25	0.49

[a] The values for human (Lu *et al.*, 1992), pig (Podschun *et al.*, 1989), and rat (Fujimoto *et al.*, 1991) were published.
[b] NA, data not available.

The expressed enzyme was characterized for its ability to metabolize typical DPD substrates such as uracil, thymine, and 5-fluorouracil (Fig. 4). The expressed enzyme exhibited inhibition of activities at high concentrations for all substrates examined, typical of ping pong reaction kinetics as previously shown for purified pig DPD (Podschun *et al.*, 1989). The K_m values obtained (Table II) were of similar magnitude to the values published for the purified pig (Podschun *et al.*, 1989), human (Lu *et al.*, 1992) and rat enzymes (Fujimoto *et al.*, 1991). The V_{max} values of expressed DPD were about 3–5-fold lower than the purified pig enzyme reflecting the fact that the expressed DPD was only partially purified. However, these data establish that the expressed enzyme reflects the properties of the purified pig liver DPD. Thus, *E. coli* should prove useful for examining any enzymatic variants obtained through screening DPD-deficient individuals and for preparing large amounts of intact holoenzyme for physycochemical analysis.

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